REMARKS/ARGUMENTS

Status of the Claims

Claims 12-19 were rejected. Claims 1-11 were previously cancelled without prejudice or disclaimer. Applicants reserve the right to pursue these claims in a continuation or divisional application. Claims 12-19 are pending in the present application.

The Rejection of Claim 12 Under 35 U.S.C. § 102 Should Be Withdrawn

Claim 12 was rejected under 35 U.S.C. § 102(b) as being anticipated by Greenwald *et al.* (1996) *Bioconjugate Chem.* 7:638-641. This rejection is respectfully traversed.

Claims 12 is drawn to a method of preparing a chemically modified hemoglobin solution that is substantially free of contaminants comprising dissolving an activated PEG (aPEG) in a solvent suitable for addition to hemoglobin and in which the aPEG is stable, filtering the aPEG solution to substantially reduce the level of contaminants, and combining the resulting filtered aPEG solution with a hemoglobin solution. Thus, the claimed methods require that the aPEG must first be dissolved in an appropriate solvent and then filtered to substantially reduce contaminants before using the filtered aPEG solution to chemically modify hemoglobin. A critical step of the claimed invention is the combination of the filtered aPEG solution with a hemoglobin solution. That is, the final aPEG solution must be filtered prior to combining it with the hemoglobin solution. As noted previously, while contaminants such as endotoxin can be removed after PEGylation, purification following chemical modification of the hemoglobin solution results in undesirable changes to the protein composition (e.g., removal of antioxidant enzymes associated with the PHP complex) or even in destruction of the product (page 3, lines 1-3). Therefore, combination of a hemoglobin solution with a filtered aPEG solution is critical to obtaining a final hemoglobin product that is substantially free of contaminants.

Contrary to the Examiner's assertions, a *prima facie* case of anticipation under 35 U.S.C. § 102 has not been established. According to the Federal Circuit, "anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration." *W.L. Gore & Assocs. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983). Greenwald *et*

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al. teach a method for preparing a chemically modified hemoglobin solution comprising combining a hemoglobin solution with an aPEG solution, specifically PEG thiazolidine-2-thione (T-PEG) via a liquid-liquid addition process. In contrast to the claimed methods, the cited reference does not teach filtering the final aPEG solution to substantially reduce contaminant levels and then combining the resulting filtered aPEG solution with the hemoglobin solution.

The Examiner maintains that the T-PEG solution of Greenwald *et al.* is subject to filtration prior to adding it to the hemoglobin solution. The filtration step referred to by the Examiner, however, occurs during the synthesis of the crystallized, solid T-PEG powder and is not equivalent to the required step of filtering the final aPEG solution recited in claim 12. In accordance with the methods of Greenwald *et al.*, the aPEG is generated in a solvent, filtered, and then recrystallized as a powder. See page 639, right column. The solid aPEG is then dissolved in an aqueous sodium phosphate/sodium chloride buffer solution, and the aPEG/buffer solution, *without any filtration*, is combined with the hemoglobin solution. See page 640, left column. Thus, the final aPEG solution of the cited reference is not filtered prior to combining it with hemoglobin, and Greenwald *et al.* do not disclose combining a filtered aPEG solution with a hemoglobin solution. The reference does not teach each element of claim 12 and, therefore, is not anticipatory.

Claim 12 further recites that the aPEG is dissolved in a solvent *suitable for addition to a hemoglobin solution and in which said aPEG is stabile*; filtering the dissolved aPEG; and, combining the filtered aPEG solution with the hemoglobin solution. Greenwald *et al.* do not teach this method. As explained on page 9, lines 25-31 of the specification, a solvent suitable for addition to a hemoglobin solution is one that does not significantly degrade or denature the polypeptide in solution. The T-PEG filtrate that is produced by the method of Greenwald *et al.* does not satisfy this requirement. In fact, following filtration Greenwald *et al.* remove the solvent present in the filtrate by distillation and must then dissolve the T-PEG into a sodium phosphate buffer before addition to the hemoglobin solution. Accordingly, contrary to the method steps recited in claim 12, the aPEG filtrate produced by the methods of Greenwald *et al.* is 1) not "suitable for addition to a hemoglobin solution" and 2) is not combined with the

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hemoglobin solution. The claimed method is not taught by the reference, and the rejection should be withdrawn.

Furthermore, although Greenwald *et al.* do not teach filtering the aPEG solution and combining the filtered aPEG solution with hemoglobin as required by the present claims, even if the filtration step cited by the Examiner was applied to the final aPEG solution, there is no evidence in the reference that this filtration step would be sufficient to substantially reduce the level of contaminants. Greenwald *et al.* only state that the reaction mixture is subject to "filtration" and do not disclose the type of filter that is actually used. See page 639, right hand column. It is unclear from this limited description that the filter employed would substantially reduce the level of contaminants of any solution. A person skilled in the art of preparing aPEGs would interpret this step as involving minimal filtration to remove insoluble material through a sintered glass filter or a paper filter. See, for example, Abuchowski *et al.* (1984) *Cancer Biochem. Biophys.* 7:175-186. In contrast, the present specification indicates that aPEG solutions that are substantially reduced in contaminants have significant reductions in the levels of, for example, bioburden, endotoxin, and particulates, are "noninfectious" (page 8, line 5), and are "characterized by not inducing pathophysiological effects characteristic of the presence of contaminants upon *in vivo* administration to a subject" (page 7, lines 30-31). Contrary to the Examiner's conclusions, Greenwald's use of an undisclosed filter would not necessarily substantially reduce the level of contaminants, as defined in the specification. A person skilled in the art would appreciate that many filters and filtration devices would be unable to sterilize a solution to the point of rendering the solution noninfectious and suitable for *in vivo* administration without inducing pathophysiological effects. Thus, Applicants respectfully disagree with the Examiner's assertion that the filtration of Greenwald *et al.* "necessarily reduces the levels of contaminants substantially" (page 4, lines 4-5, Office Action mailed July 15, 2005). Applicants note, however, that the actual filtration used by Greenwald *et al.* is moot because the reference simply does not teach filtering the aPEG solution and combining the filtered aPEG solution with hemoglobin, as recited in the present claims.

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Accordingly, for the reasons presented above, the reference does not teach each and every limitation of claim 12, and anticipation under 35 U.S.C. § 102(b) has not been established. Applicants respectfully request that the rejection of claim 12 be withdrawn.

The Rejection of the Claims Under 35 U.S.C. § 103 Should Be Withdrawn

Claim 13 was rejected under 35 U.S.C. § 103 as being unpatentable over Greenwald *et al.* as applied to claim 12 in further view of Talarico *et al.* (2000) *Biochim. Biophys. Acta* 1476:53-65. This rejection is respectfully traversed.

Dependent claim 13 encompasses the limitations of claim 12 and further requires that the aPEG is polyoxyethylene (POE). As discussed above, Greenwald *et al.* do not teach or suggest filtering the final aPEG solution to substantially reduce contaminants and combining the filtered solution with hemoglobin. In fact, this reference only describes filtering a reaction mixture during the synthesis of a powdered aPEG and does not suggest filtering the final aPEG solution. The solid aPEG of Greenwald *et al.* is dissolved in a sodium phosphate/sodium chloride buffer, and the aPEG solution is not filtered at all prior to combining it with hemoglobin.

Talarico *et al.* teach a method of producing a PEGylated hemoglobin composition comprising modifying a pyridoxalated stroma-free hemoglobin with an aPEG, specifically POE, followed by purification of the modified hemoglobin solution to remove residual reactants and contaminants. In contrast to the claimed method, the cited reference does not teach first dissolving POE in a solvent suitable for addition to hemoglobin in which the POE is stable and then filtering the POE solution prior to combining the filtered POE solution with hemoglobin.

While Greenwald *et al.* do not teach or suggest using POE to modify hemoglobin, the Examiner maintains that it would have been *prima facie* obvious to one of skill in the art to combine the cited references and replace T-PEG with POE in the method of Greenwald *et al.* to arrive at the method of claim 13. Applicants respectfully disagree with the Examiner's conclusions.

A *prima facie* case of obviousness requires some suggestion to combine the cited references to arrive at the claimed invention and a reasonable expectation of success in such a combination. First, although Greenwald *et al.* combine an (unfiltered) aPEG solution with

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hemoglobin, prior to the present invention aPEGs were typically added to hemoglobin in a powdered form due to their instability in water. In fact, Greenwald *et al.* expressly state that one advantage of T-PEG over other aPEGs is that T-PEG is "relatively stable in aqueous solutions at room temperature, making possible liquid-liquid additions rather than the typical solid addition of aPEG to protein solutions." See page 641, left column. Greenwald *et al.* actually demonstrate that N-hydroxysuccinimidyl esters of PEG, such as POE, are unstable in aqueous solutions. See Table 1 (indicating a half-life of hydrolysis for SCM-PEG of only 0.75 minutes). As such, the reference *teaches away* from using other aPEGs, including POE, in the method of Greenwald *et al.* because other aPEGs do not exhibit the same advantageous properties as T-PEG. Therefore, contrary to the Examiner's assertions, one would not have been motivated to combine the method of Greenwald *et al.* with the POE of Talarico *et al.* to produce the method of claim 13 with a reasonable expectation of success.

Furthermore, although there is no motivation to combine the cited references, even if combined, the references would not allow one of skill in the art to practice the method of claim 13. As discussed above, neither reference cited by the Examiner teaches or suggests filtering an aPEG solution to substantially reduce contaminant levels and combining the filtered aPEG solution with hemoglobin, critical steps in the present method. In addition, the claims expressly recite that the aPEG is dissolved in "a solvent suitable for addition to a hemoglobin solution and *in which said aPEG is stable*." Because POE is not stable in the aqueous sodium phosphate/sodium chloride buffer solution of Greenwald *et al.*, as indicated in Table 1 of the reference, the POE taught by Talarico *et al.* could not replace T-PEG in the method of Greenwald *et al.* to produce the method of claim 13. Accordingly, the combination of cited references could not have placed the invention of claim 13 in the hands of the public, and a *prima facie* case of obviousness has not been established.

Claim 14 was rejected under 35 U.S.C. § 103 as being unpatentable over Greenwald *et al.* as modified by Talarico *et al.* as applied to claim 13 in further view of Woghiren *et al.* (1993) *Bioconjugate Chem.* 4:314-318, Blume *et al.* (1990) *Biochim. Biophys. Acta* 1029:91-97, or Maraganore *et al.* (U.S. Patent No. 5,256,559). This rejection is respectfully traversed.

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Dependent claim 14 further requires that POE is dissolved in a solvent selected from the group consisting of ethanol, methanol, and acetonitrile. Although neither Greenwald *et al.* nor Talarico *et al.* disclose dissolving an aPEG in ethanol, methanol, or acetonitrile, the Examiner maintains that it would have been *prima facie* obvious for one skilled in the art to dissolve POE in an organic solvent in view of the teachings of Woghiren *et al.*, Blume *et al.*, or Maraganore *et al.* and use this POE solution in the method of Greenwald *et al.* to produce the method of claim 14. Applicants respectfully disagree with the Examiner's conclusions.

First of all, contrary to the Examiner's assertions, Woghiren *et al.* do not disclose dissolving an aPEG in methanol. The cited reference teaches the use of methanol in an alcoholysis step during the synthesis of PEG-SH, not for the dissolution of the final aPEG as required by the present claims. The Blume *et al.* reference discloses an aPEG solution in chloroform/methanol for use in the production of PEGylated liposomes but does not teach or suggest using this aPEG solution to modify proteins. Maraganore *et al.* disclose a method for preparing PEGylated hirudin and indicate that the aPEG is preferably in an organic solvent or a buffered solution. None of the cited references teaches or suggests a method for chemically modifying hemoglobin or discusses using POE as the aPEG to practice the disclosed methods.

As indicated above, the present claims recite that the aPEG is dissolved in a solvent suitable for addition to a hemoglobin solution, which, as defined in the specification, requires that the solvent does not significantly denature or degrade hemoglobin. See page 9, lines 28-30. While the chloroform/methanol solution taught by Blume *et al.* is appropriate for dissolution of lipids or peptides, proteins such as hemoglobin are denatured in this solvent and, therefore, chloroform/methanol could not be used to practice the claimed methods. In addition, none of the references cited in the present Office Action teaches or suggests dissolving an aPEG in a solvent that is compatible with hemoglobin, filtering this aPEG solution, and then combining the filtered aPEG solution with a hemoglobin solution.

Furthermore, as noted above, Greenwald *et al.* disclose the synthesis of a powdered aPEG and the dissolution of this solid aPEG in an aqueous sodium phosphate/sodium chloride buffer. Greenwald *et al.* do not teach or suggest dissolving the aPEG in an organic solvent, as required by claim 14. Therefore, Applicants submit that there is insufficient motivation to combine the

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cited references to arrive at the method of claim 14. Moreover, the references, even if combined, would not allow a person skilled in the art to produce the method of claim 14. None of the cited references teaches or suggests filtering an aPEG solution to substantially reduce contaminants and combining the resulting filtered aPEG solution with hemoglobin. Therefore, the references simply cannot be combined to arrive at the method of claim 14, and a *prima facie* case of obviousness has not been established.

Claims 15 and 16 were rejected under 35 U.S.C. § 103 as being unpatentable over Greenwald *et al.* as modified by Talarico *et al.*, Woghiren *et al.*, Blume *et al.*, and Maraganore *et al.* as applied to claim 14 in further view of Shorr (U.S. Patent No. 5,900,402). This rejection is respectfully traversed.

Claim 15 further requires that the filtration of the aPEG solution substantially reduces endotoxin contaminant levels present in the aPEG solution. Claim 16 further recites that the endotoxin contaminant levels are reduced by at least 500 EU/cm^2 of filter area. The Examiner asserts that claims 15 and 16 are obvious in view of the filters taught by Shorr. The cited reference teaches the use of a Sartorius Q membrane and a 0.22 micron filter to remove endotoxin from a PEGylated hemoglobin solution. The Examiner maintains that it would have been obvious to one of skill in the art to use the filters disclosed by Shorr in conjunction with the method of Greenwald *et al.* in combination with the other cited references to arrive at the methods of claim 15 and 16 for the expected benefit of removing or reducing the levels of endotoxin in the hemoglobin product.

In contrast to claims 15 and 16, however, Shorr only discloses the use of filters to remove endotoxin contaminants from the final chemically modified hemoglobin solution. As indicated above, the hemoglobin solution produced by the claimed methods cannot be purified after chemical modification because such filtration would disrupt, or even destroy, the hemoglobin composition. Therefore, the aPEG solution must be filtered to substantially reduce endotoxin contaminants before the filtered aPEG solution is combined with hemoglobin in order to practice the methods of claim 15 and 16. Furthermore, Shorr does not teach or suggest filtering an aPEG solution through a filter capable of removing endotoxin or through <u>any</u> filter. The mere fact that

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Shorr used a filter to remove endotoxin contaminants from a hemoglobin solution is no indication that one of skill in the art would have been motivated to dissolve an aPEG in a solvent in which it is stable, filter the aPEG solution through a filter of any type, and then use the filtered aPEG solution to modify hemoglobin. Moreover, given that Greenwald *et al.* do not teach or suggest filtering an aPEG solution and then combining the filtered aPEG solution with hemoglobin, the references, even if combined, would not allow one skilled in the art to arrive at the methods of claim 15 and 16. Therefore, claims 15 and 16 are not obvious.

Claims 17 and 18 were rejected under 35 U.S.C. § 103 as being unpatentable over Greenwald *et al.* as modified by Talarico *et al.*, Woghiren *et al.*, Blume *et al.*, Maraganore *et al.*, and Shorr (U.S. Patent No. 5,900,402) as applied to claim 16 in further view of Nho *et al.* (U.S. Patent No. 5,234,903). This rejection is respectfully traversed.

Dependent claims 17 and 18 further require filtering the aPEG solution through a 0.2 micron nylon filter. Nho *et al.* teach using a 0.2 micron Zetapor® nylon filter to sterilize a chemically modified hemoglobin solution and to render it substantially endotoxin-free. In light of this and the other cited references, the Examiner maintains that it would have been obvious to one of skill in the art to replace the filter of Shorr with the 0.2 micron nylon filter of Nho *et al.* and to use this 0.2 micron filter to filter an aPEG solution to substantially reduce contaminant levels in accordance with the methods of claim 17 and 18. Neither Nho *et al.* nor any of the references cited by the Examiner teach or suggest filtering an aPEG solution through a filter of any size and combining the filtered aPEG with a hemoglobin solution, as required by the present claims. Moreover, the mere fact that Nho *et al.* use a 0.2 micron nylon filter to remove contaminants from a chemically modified hemoglobin solution is no indication that one of skill in the art would have been motivated to use such a filter, or a filter of any size, to filter an aPEG solution to substantially reduce contaminant levels and then combine the filtered aPEG solution with hemoglobin. Therefore, claims 17 and 18 are not obvious in view of the cited references.

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Claim 19 was rejected under 35 U.S.C. § 103 as being unpatentable over Greenwald *et al.* as modified by Talarico *et al.*, Woghiren *et al.*, Blume *et al.*, Maraganore *et al.*, Shorr, and Nho *et al.*. This rejection is respectfully traversed.

Dependent claim 19 further requires that the steps of filtering the aPEG and combining the filtered aPEG with hemoglobin are aseptically joined. The Examiner maintains that Nho *et al.* taught accomplishing the method steps of preparing a chemically modified hemoglobin solution under sterilizing conditions and therefore one skilled in the art would have been motivated to produce the method of claim 19 to achieve a highly sterile product for *in vivo* administration. Applicants respectfully disagree with the Examiner's conclusions.

First of all, the Examiner expressly acknowledges that the Nho *et al.* reference does not "disclose that the filtering and combining steps are aseptically joined" (page 7, lines 23-24, Office Action mailed July 15, 2005). In fact, Nho *et al.* do not even suggest filtering an aPEG solution and therefore also could not have taught or suggested aseptically coupling the filtering of an aPEG solution with the step of combining the filtered aPEG solution with a hemoglobin solution. Moreover, contrary to the Examiner's assertions, the specific sections cited in the Nho *et al.* patent (i.e., sections 5.1.1.1; 5.1.4; 6.1.5; 6.2, 10.1.5; and 10.2) do not teach or suggest performing each step during the preparation of the modified hemoglobin solution under sterile conditions. In fact, these sections highlight the fact that the primary method for ensuring sterility of the hemoglobin product produced by the method of Nho *et al.* is by filtering the final PEG-hemoglobin solution through a 0.2 micron filter, and not by maintaining sterility at each step during the preparation of the modified hemoglobin. Furthermore, the mere recognition in the art of a need for purified hemoglobin solutions is no indication that a person skilled in the art would have been motivated to dissolve an aPEG in solvent suitable for addition to a hemoglobin solution and in which the aPEG is stable, to filter the aPEG solution to substantially reduce the level of contaminants, and to combine the filtered aPEG solution with a hemoglobin solution, wherein the filtering and combining steps are aseptically joined.

And finally, the references cited by the Examiner cannot be combined to produce the method of claim 19. None of the references teaches or suggests the required steps of filtering the aPEG solution and combining the resulting filtered aPEG solution with a hemoglobin solution.

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Moreover, the requirement for aseptic coupling of the filtering and combining steps is also not taught by or suggested in any of the references cited by the Examiner. As such, the combination of references could not have placed the method of claim 19 in the hands of the public. Therefore, Applicants respectfully submit that claim 19 is not obvious and request that the rejection be withdrawn.

In summary, there is insufficient motivation to combine the cited references to arrive at the methods of claims 13-19. Furthermore, the references, even if combined, would not allow a person skilled in the art to produce the claimed methods, particularly since none of the cited references teaches or suggests the critical steps of dissolving an aPEG in a solvent suitable for addition to hemoglobin and in which the aPEG is stable, filtering the aPEG solution to substantially reduce contaminants, and combining the filtered aPEG solution with a hemoglobin solution.

For the reasons presented above, the Examiner has failed to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully submit that the claimed methods for producing a chemically modified solution substantially free of contaminants are not obvious in view of the cited references and request that the rejection of claims 12-19 under 35 U.S.C. § 103(a) be withdrawn.

CONCLUSION

The Examiner is respectfully requested to withdraw the rejections and allow claims 12-19. In view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of

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this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

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